

WHAT IS CLAIMED IS:

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1. A nucleic acid primer having a 5' end and a 3' end, comprising:
- (a) a first region containing the 5' end of the primer and an immobilization attachment site; and
- (b) a second region containing the 3' end of the primer and a chemically cleavable site, wherein the 3' end is capable of being extended by an enzyme.
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2. The primer of claim 1, wherein the chemically cleavable site is located at or within about five nucleotides from the 3' end of the primer.
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3. The primer of claim 2, wherein the second region of the primer comprises a single nucleotide.
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4. The primer of claim 3, wherein the second region comprises a ribonucleotide.
5. The primer of claim 1, wherein the chemically cleavable site comprises a modified base, a modified sugar, or a chemically cleavable group incorporated into the phosphate backbone.
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6. The primer of claim 5, wherein the chemically cleavable site comprises a modified sugar.
7. The primer of claim 1, where the chemically cleavable site is selected from the group consisting of dialkoxysilane, 3'-(S)-phosphorothioate, 5'-(S)-phosphorothioate, 3'-(N)-phosphoramidate, 5'-(N)phosphoramidate, and ribose.
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8. The primer of claim 7, wherein the chemically cleavable site is 3'-(S)-phosphorothioate or 5'-(S)-phosphorothioate.
9. The primer of claim 1, wherein the enzyme is a DNA polymerase.
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10. The primer of claim 1, wherein the enzyme is a ligase.

11. The primer of claim 1, further comprising a solid support attached to the immobilization attachment site.

12. The primer of claim 11, wherein the immobilization attachment site is attached to an
5 intervening spacer arm bound to the solid support.

13. The primer of claim 12, wherein the intervening spacer arm is six or more atoms in length.

10 14. The primer of claim 11, wherein the solid support is selected from the group consisting of glass, silicon, polystyrene, aluminum, steel, iron, copper, nickel, silver and gold.

15 15. The primer of claim 11, wherein the solid support comprises a functionality selected from the group consisting of avidin and streptavidin.

16. The primer of claim 11, wherein the solid support comprises an antibody.

17. The primer of claim 16, wherein the antibody comprises anti-digoxigenin.

20 18. The primer of claim 1, wherein the immobilization attachment site is a substituent on one of the bases or sugars of the primer.

25 19. The primer of claim 1, wherein said immobilization attachment site is biotin or digoxigenin.

20. The primer of claim 1, wherein the immobilization attachment site comprises a single-stranded nucleic acid.

30 21. The primer of claim 20, further comprising a solid support, wherein the single stranded nucleic acid is complementary to an intermediary oligonucleotide bound to the solid support and wherein the primer is attached to the solid support by hybridization of the immobilization attachment site to the intermediary oligonucleotide.

22. A kit for analyzing at least one target nucleic acid, comprising:

- (a) a first primer, having a 5' end and a 3' end, complementary to a first target nucleic acid, wherein the first primer has a first region containing the 5' end of the first primer, and a second region containing the 3' end of the first primer and a chemically cleavable site; and
- (b) a second primer complementary to an extension product of the first primer.

23. The kit of claim 22, wherein the second primer further comprises an immobilization attachment site.

24. The kit of claim 23, wherein the immobilization attachment site is selected from the group consisting of biotin and digoxigenin.

25. The kit of claim 22, wherein the first primer further comprises an immobilization attachment site.

26. The kit of claim 25, wherein the immobilization attachment site is a substituent on a base or sugar of the first primer.

27. The kit of claim 25, wherein the immobilization attachment site comprises an antigen.

28. The kit of claim 25, wherein the immobilization attachment site is selected from the group consisting of biotin and digoxigenin.

29. The kit of claim 22, further comprising an enzyme for extending the first and second primers.

30. The kit of claim 29, wherein the enzyme is a DNA polymerase.

31. The kit of claim 29, wherein the enzyme is a ligase.

32. The kit of claim 22, wherein the chemically cleavable site comprises a modified base, a modified sugar, or a chemically cleavable group incorporated into the phosphate backbone.

33. The kit of claim 32, wherein the chemically cleavable site comprises a modified sugar.

34. The kit of claim 22, where the chemically cleavable site is selected from the group consisting of dialkoxysilane, 3'-(S)-phosphorothioate, 5'-(S)-phosphorothioate, 3'-(N)-phosphoramidate, 5'-(N)phosphoramidate, and ribose.

35. The kit of claim 34, wherein the chemically cleavable group comprises 3'-(S)-phosphorothioate or 5'-(S)-phosphorothioate.

36. The kit of claim 25, further comprising a solid support.

37. The kit of claim 36, wherein the solid support is capable of being attached to the immobilization attachment site.

38. The kit of claim 37, wherein the immobilization attachment site is capable of being attached to an intervening spacer arm bound to the solid support.

39. The kit of claim 38, wherein the intervening spacer arm is six or more atoms in length.

40. The kit of claim 36, wherein the solid support is selected from the group consisting of glass, silicon, polystyrene, aluminum, steel, iron, copper, nickel, silver and gold.

41. The kit of claim 36, wherein the solid support comprises a functionality selected from the group consisting of avidin and streptavidin.

42. The kit of claim 36, wherein the solid support comprises an antibody.

43. The kit of claim 42, wherein the antibody comprises anti-digoxigenin.

44. The kit of claim 36, wherein the immobilization attachment site comprises a single-stranded nucleic acid complementary to an intermediary oligonucleotide bound to the solid support.

45. The kit of claim 22, further comprising a reagent capable of cleaving the chemically cleavable site.

46. The kit of claim 45, wherein the reagent is selected of 2-iodoethanol, 2,3-epoxy-1-propanol, silver, and fluoride.

47. The kit of claim 25 adapted for analyzing more than one target nucleic acid, further comprising:

- (a) a third primer, having a 5' end and a 3' end, complementary to a second target nucleic acid, wherein the third primer has a first region containing the 5' end of the third primer and an immobilization attachment site, and a second region containing the 3' end of the third primer and a chemically cleavable site; and
- (b) a fourth primer complementary to an extension product of the third primer strand, wherein the masses of extension segments generated by extending the primers in the presence of the target nucleic acids and cleaving at the cleavable sites of the first and third primers are distinguishable by mass spectrometry.

48. The kit of claim 47, wherein the cleavable sites of the first and third primer are located at differing locations relative to the 3' ends of the first and third primers.

49. A kit for simultaneously analyzing more than one target nucleic acid, comprising:

- (a) a plurality of first primers, each having a 5' end and a 3' end, complementary to a plurality of target nucleic acids, wherein the first primers each have a first region containing the 5' end, and a second region containing the 3' end of the respective first primer and a chemically cleavable site; and
- (b) a plurality of second primers complementary to a plurality of extension products of the plurality of first primers, wherein the masses of extension segments generated by extending the primers in the presence of the target nucleic acids and cleaving at the cleavable sites of the first primers are distinguishable by mass spectrometry.

50. The kit of claim 49, wherein the location of the cleavable sites of the first primers relative to their respective 3' ends are selected to distinguish the masses of extension segments generated by extending the first and second primers in the presence of the plurality of target nucleic acids and cleaving at the cleavable sites of the first primers.

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51. The kit of claim 49, wherein at least one first primer further comprises an immobilization attachment site.

52. The kit of claim 49, wherein at least one second primer further comprises an
10 immobilization attachment site.

53. The kit of claim 49, further comprising an enzyme for extending the first and second primers.

15 ~~54. A method for determining the size of more than one primer extension product, comprising:~~

~~(a) hybridizing a plurality of primers, each having a 5' end and a 3' end, with more than one target nucleic acid, wherein each of said primers~~

~~(i) is complementary to at least one target nucleic acid;~~

~~(ii) has a first region containing the 5' end of the primer, and~~

~~(iii) has a second region, containing the 3' end of the primer and a cleavable site, wherein the 3' end is capable of being extended by an enzyme;~~

~~(b) extending the primers with the enzyme to generate a polynucleotide mixture containing more than one extension product;~~

25 ~~(c) cleaving more than one extension product at its respective cleavable site to release more than one extension segment, wherein the location of the cleavable site of at least two primers is selected to increase the mass difference between their respective extension segments; and~~

- (d) sizing the released extension segments by mass spectrometry, whereby said cleaving is effective to increase the read length of the extension segments relative to the read length of the products of step (b).

5 55. The method of claim 54, wherein the first region of at least one of said primers comprises an immobilization attachment site.

56. The method of claim 55, wherein one or more extension products is immobilized onto a solid support.

10 57. The method of claim 56, further comprising washing the extension product after said immobilizing and prior to said cleaving.

58. The method of claim 54, wherein at least one target nucleic acid is DNA.

15 59. The method of claim 54, wherein at least one target nucleic acid is RNA.

60. The method of claim 54, wherein the target nucleic acids are immobilized.

20 61. The method of claim 60, wherein the target nucleic acids are immobilized prior to said extending.

62. The method of claim 61, wherein the target nucleic acids are immobilized after said cleaving.

25 63. The method of claim 54, wherein at least one cleavable site is a blocking nucleotide capable of blocking a 5' to 3' enzyme-promoted digestion, and wherein said cleaving is carried out by digesting the first region of at least one primer with an enzyme having a 5' to 3' exonuclease activity.

30 64. The method of claim 54, wherein at least one cleavable site comprises a modified base, a modified sugar, or a chemically cleavable group incorporated into the phosphate backbone.

65. The method of claim 64, wherein at least one cleavable site comprises a modified sugar.

66. The method of claim 54, where at least one cleavable site is selected from the group consisting of ~~dialkoxysilane, 3'-(S)-phosphorothioate, 5'-(S)-phosphorothioate,~~

5 ~~3'-(N)-phosphoramidate, 5'-(N)phosphoramidate, uracil, and ribose.~~

67. The method of claim 66, wherein at least one cleavable site comprises 3'-(S)-phosphorothioate or 5'-(S)-phosphorothioate.

10 68. The method of claim 54, where said sizing is by time-of-flight mass spectrometry.

~~69. A method for determining presence of a polymorphism, comprising:~~

~~(a) hybridizing a primer, having a 5' end and a 3' end, with a target nucleic acid suspected of containing a polymorphism, wherein said primer has a first region containing the 5' end of the primer and a second region containing the 3' end of the primer and a cleavable site;~~

~~(b) extending the 3' end of the primer with a polymerase in the presence of a nucleotide to generate an extension product;~~

~~(c) cleaving said extension product at the cleavable site to release an extension segment;~~

~~(d) sizing the extension segment by mass spectrometry, whereby said cleaving is effective to increase the read length of the extension segment relative to the read length of the product of step (b); and~~

~~(e) identifying any added nucleotides.~~

25 70. The method of claim 69, wherein the first region of the primer further comprises an immobilization attachment site and the extension product is immobilized onto a solid support at the immobilization attachment site prior to the cleaving step.

30 71. The method of claim 70, further comprising washing the extension product after said immobilizing and prior to said cleaving.

72. The method of claim 69, wherein the nucleotide is selected from the group consisting of a deoxynucleotide, a chain-terminating nucleotide and a derivative thereof.

73. The method of claim 72, wherein the nucleotide is a chain-terminating nucleotide.

74. The method of claim 73, wherein the chain-terminating nucleotide is a dideoxynucleotide.

75. The method of claim 74, wherein the dideoxynucleotide is selected from the group consisting of ddATP, ddTTP, ddUTP, ddGTP, ddITP and ddCTP.

76. The method of claim 73, wherein the chain-terminating nucleotide is mass modified.

77. The method of claim 69, wherein the target nucleic acid is DNA.

78. The method of claim 69, wherein the target nucleic acid is RNA.

79. The method of claim 69, wherein the target nucleic acid is immobilized.

80. The method of claim 79, wherein the target nucleic acid is immobilized prior to said extending.

81. The method of claim 79, wherein the target nucleic acid is immobilized after said extending.

82. The method of claim 69, wherein the cleavable site is a blocking nucleotide capable of blocking 5' to 3' enzyme-promoted digestion, and where said cleaving is carried out by digesting the first region of the primer with an enzyme having a 5' to 3' exonuclease activity.

83. The method of claim 82, wherein the enzyme is selected from the group consisting of T7 gene 6 exonuclease and phosphodiesterase.

84. The method of claim 82, wherein the blocking nucleotide is selected from the group consisting of phosphorothioate, methyl phosphonate, phosphotriester, and peptide nucleic acid.

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85. The method of claim 69, where the cleavable site is selected from the group consisting of dialkoxysilane, 3'-(S)-phosphorothioate, 5'-(S)-phosphorothioate, 3'-(N)-phosphoramidate, 5'-(N)-phosphoramidate, uracil, and ribose.

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86. The method of claim 69, wherein said sizing is by time-of-flight mass spectrometry.

87. The method of claim 69, wherein a plurality of primers are hybridized to more than one target nucleic acid and the location of the cleavable site contained within the primers is varied to

10 increase the mass difference between their respective extension segments.